Immunogenicity of Monoclonal Antibody Products

March 29, 2006

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Outline

- **Factors influencing the immunogenicity of monoclonal antibodies**
- **Assays for antibodies to monoclonal antibody products**
- **Example**
- **Issues for products with special use**

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Antibody Purity -1

- **Impurities (cell substrate, media components) that co-purify with the antibody**
  - **Can be directly immunogenic**
  - **Can act as an adjuvant**
- **Product-related impurities**
  - **Fragments**
  - **Aggregates**

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Antibody Purity -2

- **Antibodies Used as Ancillary Products**
  - **Cell depletion**
    - **in solution**
    - **immobilized**
  - **Affinity chromatography**
    - **purification**

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Modified Antibodies

- **Conjugates of mAb to drugs, toxins, chelators or antibody fusion products**
  - **immunogenicity of the added substance**
  - **creation of new antigenic determinants at the site of conjugation or fusion**
- **Antibody fragments**
  - **exposure of new antigenic determinants**

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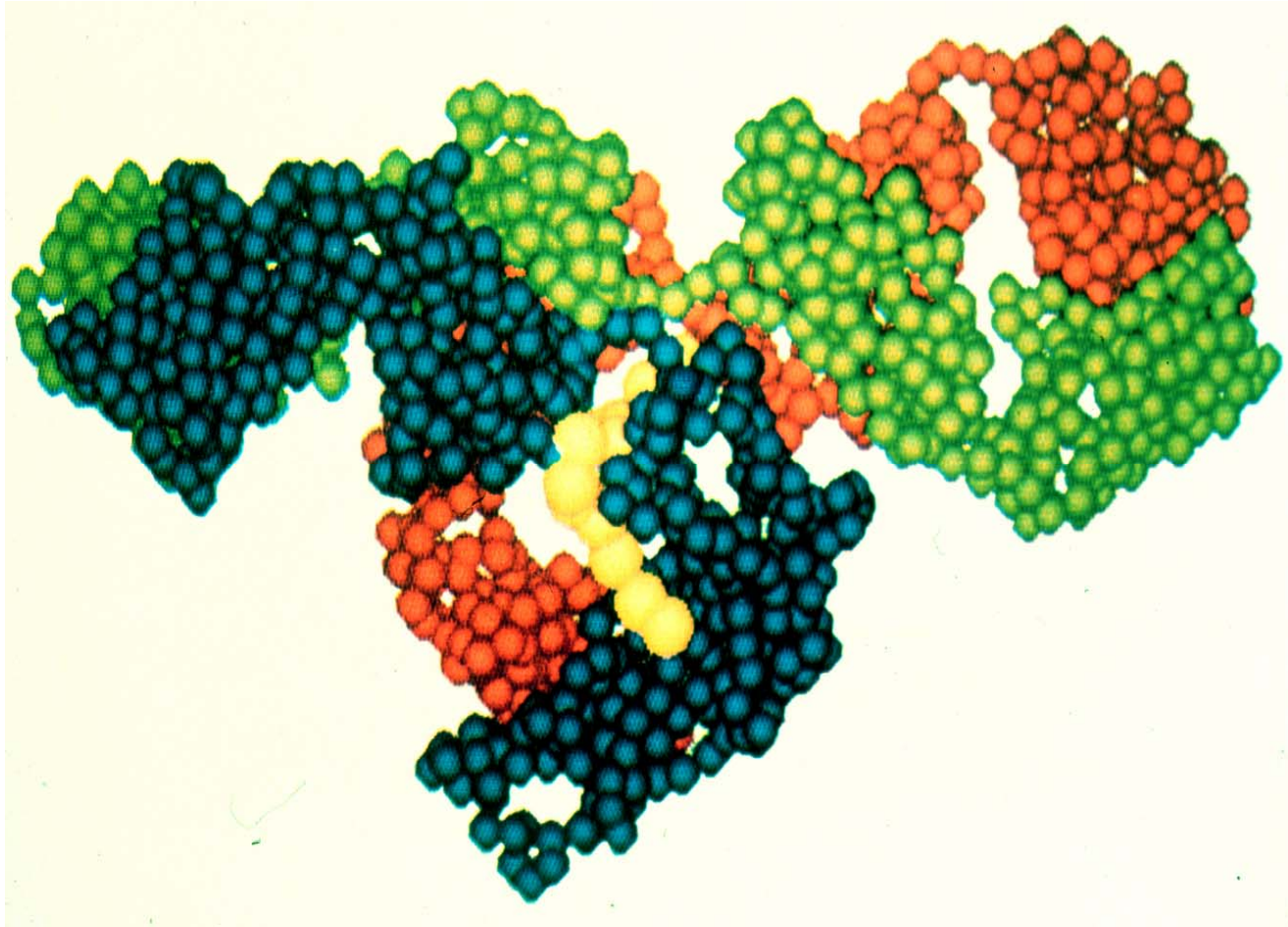
Antibody 3-Dimensional Structure



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Antibodies are Inherently Immunogenic

- **All antibodies are unique and potentially immunogenic (idiotype)**
  - **Immunogenicity must be evaluated for each product**
  - **Comparison of immunogenicity across products may be problematic**
- **Fc region can be immunogenic (especially murine)**
  - **HAMA, HACA, HAHA**
  - **Allotypic determinants**
  - **New antigenic determinants in engineered molecules**

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Clinical Factors Influencing the Immunogenicity of mAbs- 1

- **Patient population**
  - **Genetic background**
  - **Autoimmune diseases**
- **Pre-existing antibodies**
  - **RF, IgM anti-IgG will react with some products**
- **Intercurrent illnesses disrupt the distribution of proteins**
  - **Kidney and liver disease**
  - **Disruption of the blood/brain barrier**

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Clinical Factors Influencing the Immunogenicity of mAbs- 2

- **Concomitant medications**
  - **Chemotherapy**
  - **Immunosuppressive drugs**
- **Dose - immunogenicity increases with dose and frequency of administration**
  - **Exception may be high dose tolerance**
- **Route of administration**
  - **SC generally more immunogenic than IM**

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Consequences of Anti-mAb Antibody Formation - 1

- **Limits usefulness of pre-clinical studies in animals**
- **Effects on bioavailability**
  - **Increase, due to increase in size if not neutralizing**
  - **Decrease, due to increased clearance**
  - **Changes in initial volume of distribution at first time point due to removal of the complex from the circulation**
- **Loss of effectiveness**
  - **Neutralizing antibodies (anti-Id), loss of imaging quality**
  - **Persistent antibody leads to loss of activity (Tysabri®, 10% of patients had antibody and 6% were persistently positive)**
    - **Reduced serum levels of Tysabri®**
    - **Relapse rate in persistently-antibody positive patients receiving Tysabri® was the same as in patients receiving placebo**

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Consequences of Anti-mAb Antibody Formation - 2

- **Adverse events**
  - **Injection site reactions**
  - **Systemic reactions ranging from mild to life threatening**
    - **Hypersensitivity, urticaria, rigors, nausea, vomiting, flushing, headache**
  - **Additional infusion reactions seen in persistently-antibody positive patients (Tysabri®)**
    - **Myalgia, hypertension, dyspnea, anxiety, and tachycardia**
- **Limiting the utility of another monoclonal antibody of the same species**
- **Interference with monoclonal antibody based diagnostic tests**

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Immunogenicity of Licensed Monoclonal Antibodies (% patients with antibodies)

- Murine, n=8 (includes 1 IgM)
  - Whole antibodies
    - 2% to >80% (loss of effectiveness of OKT3 if titers > 1:1000)
  - Fab or Fab' fragments
    - <1% to 8%
- Zevalin therapeutic regimen (In^{111} or Yt^{90}-labeled murine + chimeric)
  - 4% HAMA or HACA
- Chimeric, n=5, 4/5, whole antibodies, 1 Fab
  - <1% to 13%
- Humanized, n=9, whole antibodies
  - <1% to 10%
- Human, n=1, whole antibody
  - 5%

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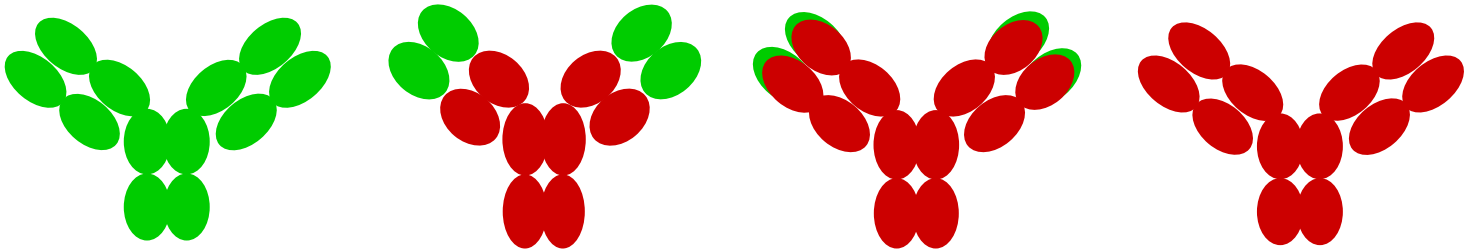
Immunogenicity Related to Fc

% of Recipients who made Anti-Product Antibodies *



Murine	Chimeric	Humanized	Human
2-80	<1-13	<1-10	5
n=9	n=5	n=9	n=1

*Data taken from Package Inserts of approved products

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Unusual Reactions

- **Remicade**
  - **Delayed reactions, 3-12 days following re-administration after 2-4 year interval from initial treatment (symptoms included myalgia, rash, fever, polyarthralgia, et al.) in 10 of 40 patients**
- **Enbrel**
  - **Recall injection site reactions (redness) in site of previous injection in 15 of 213 patients in controlled trials**

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Measurement of the Immunogenicity of Antibodies

- **Influenced by the detection assay**
  - **Sensitivity**
  - **Specificity**
- **Product specific**
  - **Idiotype**
  - **Allotype**
  - **Class, subclass**
- **Influenced by the timing of sample collection**
- **Influenced by the presence of circulating drug**

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MGAWN1 (hE16)* Product Summary

- **Humanized anti-WNV IgG1 monoclonal antibody expressed in CHO cells**
- **Binds domain III of WNV E protein**
- **Neutralizing epitope (16 residues) is conserved**
  - **Epitope is intact in 136 of 144 sequenced isolates (including all 74 North American isolates)**
  - **Neutralizes all WNV isolates tested (11 from lineage 1; 2 from lineage 2)**
- **Manufactured at MacroGenics, Inc.**

*Collaboration with Michael Diamond, M.D.
Washington University, St. Louis

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Human anti-Human Antibody Detection: “Bridging” Format

- Coat plate with MGAWN1, Fab or Fc fragment
- Incubate with sample or irrelevant human IgG1
- Detect with biotinylated MGAWN1 + SA-HRP
- Positive controls
  - Goat anti-MGAWN1
    - Whole molecule
    - Fv-specific fraction
  - Anti-Idiotypic mAb(s)

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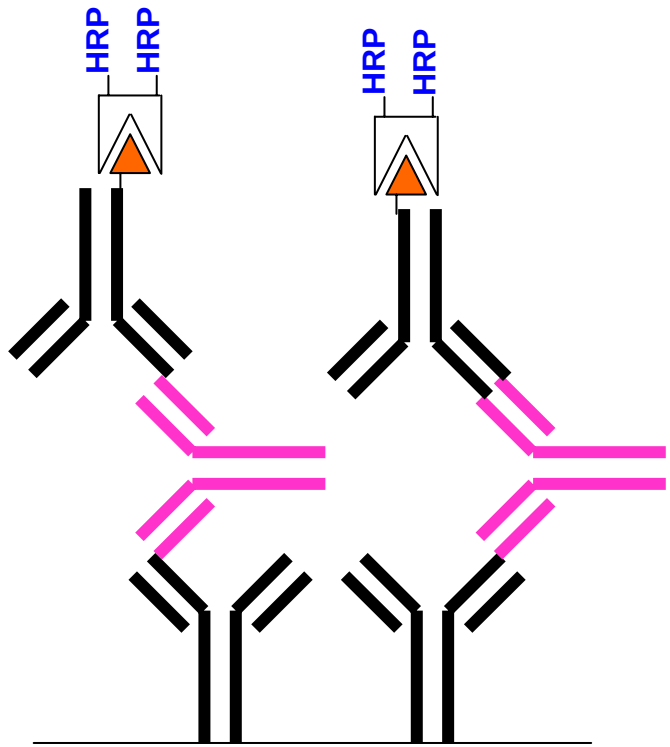
Human anti-Human Antibody Detection: “Bridging” Format



⇐ Streptavidin-HRP

⇐ MGAWN1-biotin

⇐ Anti-product antibody

⇐ MGAWN1 (or Fab or Fc fragment)

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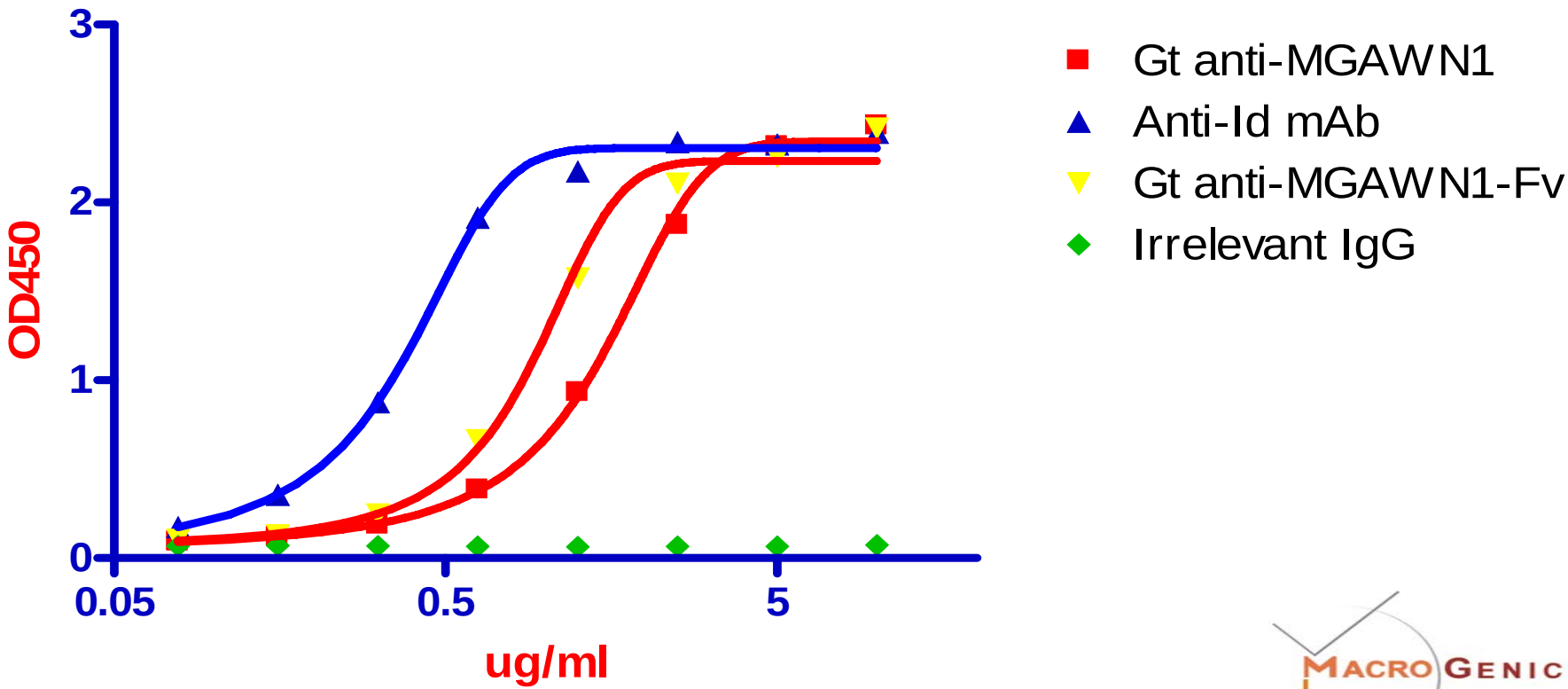
Use of “Bridging” Assay Format to Detect anti-Humanized Antibodies



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Alternative Formats

- BIAcore (real time readout)
- ECL electro chemiluminescence (homogeneous)
- Luminex/colorimetric bead array (FACS)
- Other ELISA formats
  - Fab coating/anti-human Fc detection
    - Use tetanus toxoid as a control if no positive control exists
  - Fc coating/anti-human Kappa + Lambda detection

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Special issues for Biodefense and Emerging Infection Therapeutics

- **One-time use in an emergency situation**
  - **Weigh immunogenicity issues in the context of a risk/benefit for the clinical setting**
- **May be no alternative product**
  - **If resources are not available to humanize, chimeric antibody may be adequate**
- **Formulation issues**
  - **May need room temperature (or higher) stable product**
  - **lyophilization**

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Acknowledgement

Syd Johnson, Ph.D. **Sr. Director, Antibody Engineering**

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Approved Monoclonal Antibody Products - 1

Company	USAN Name	Trade Name	Indication(s)	Approval
Ortho Biotech Products, LP	Muromonab-CD3	Orthoclone OKT3 (CD3)	Treatment of organ graft rejection	1986
Cytogen Corporation	Satumomab	Oncoscint (CEA)	Imaging colorectal/ovarian cancer	1991
Centocor, Inc.	Abciximab	ReoPro (GPIIb/IIIa)	Prevention of ischemia during PTCA	1994
DuPont Merck Pharmaceuticals	Nofetumomab	Verluma (GP40)	Imaging small cell lung cancer	1996
Cytogen Corporation	Capromab Pendetide	Prostascint (PSMA)	Imaging prostate cancer	1996
Immunomedics, Inc.	Arcitiumomab	CEA-Scan (CEA)	Imaging colorectal cancer	1996
Centocor, B.V.	Imciromab	Myoscint (cardiac myosin)	Imaging myocardial infarctions	1996

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Approved Monoclonal Antibody Products - 2

Company	USAN Name	Trade Name	Indication(s)	Approval
Genentech, Inc.	Rituximab	Rituxan (CD20)	Treatment of Non-Hodgkin's lymphoma	1997
Hoffman-LaRoche	Daclizumab	Zenapax (CD25)	Prevention of renal allograft rejection	1997
Novartis Pharmaceuticals	Basiliximab	Simulect (CD25)	Prevention of renal allograft rejection	1998
MedImmune	Palivizumab	Synagis (RSV)	Prevention of RSV hospitalization	1998
Centocor, Inc.	Infliximab	Remicade (TNFα)	Treatment of Crohn's Disease	1998
			Treatment of Rheumatoid Arthritis	1999
Genentech, Inc.	Trastuzumab	Herceptin (HER2)	Treatment of metastatic breast cancer	1998

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Approved Monoclonal Antibody Products - 3

Company	USAN Name	Trade Name	Indication(s)	Approval
Genzyme Corporation	Alemtuzumab	Campath (CD52)	Treatment of B cell CLL	2001
Wyeth-Ayerst Pharmaceuticals	Gemtuzumab	Mylotarg (CD33)	Treatment of acute myeloid leukemia	2000
IDEC Pharmaceuticals	Ibritumomab tiuxetan	Zevalin (CD20)	Treatment of relapsed or refractory NHL	2002
Abbott Laboratories	Adalimumab	Humira (TNFα)	Treatment of Rheumatoid Arthritis	2002
Genentech, Inc.	Omalizumab	Xolair (IgE)	Treatment of moderate to severe asthma	2003
Corixa Corporation	Tositumomab	Bexxar (CD20)	Treatment of Rituxan refractory NHL	2003

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Approved Monoclonal Antibody Products - 4

Company	USAN Name	Trade Name	Indication(s)	Approval
Genentech, Inc.	Efalizumab	Raptiva (CD11a)	Treatment of psoriasis	2003
Bristol-Myers Squibb	Cetuximab	Erbitux (EGFR)	Treatment of colorectal cancer	2004
Genentech, Inc.	Bevacizumab	Avastin (VEGF)	Treatment of colorectal cancer	2004
Palatin Technologies Mallinckrodt	(murinelgm) fanelesomab	NeutroSpec (99m Tc; CD15)	Imaging of atypical acute appendix	2004
Elan Phamaceuticals Biogen Idec	Natalizumab	Tysabri (α4-integrin)	Relapsing multiple sclerosis	2004

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